

## Rearing larvae of the chestnut tiger butterfly, *Parantica sita* (Kollar) (Lepidoptera, Danaidae), on artificial diets

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**Abstract** Larvae of *Parantica sita* were reared with 5 mixtures of a ready-made diet material, *Insecta F-II* (Nihon-Nosan-Kogyo Co.), and 10 or 30% dried leaf powder from three host plants, *Cynanchum caudatum*, *Metaplexis japonica*, and *Marsdenia tomentosa* (Asclepiadaceae). The larval mortalities were high in the diets with 10% leaf powder of *C. caudatum* and *M. japonica*, and 25% of the butterflies were abnormal in the diets with 30% leaf powder of *C. caudatum*. By contrast, the rates of adult emergence were high (100 and 97%) and most adults were healthy looking in the diets with 30% leaf powder of *M. japonica* and *M. tomentosa*. Since *M. japonica* is common in Japan, an artificial diet made from *M. japonica* seems to be the most convenient for the mass rearing of *P. sita*.

**Key words** Artificial diet, butterfly, mass rearing, *Parantica sita*, *Cynanchum caudatum*, *Metaplexis japonica*, *Marsdenia tomentosa*.

### Introduction

Many kinds of artificial diets for butterfly larvae have been developed to avoid such difficulties as deterioration and defoliation of host plants, and also to prevent the introduction of diseases and parasites, and to make possible mass rearing (e. g. David and Gardiner, 1965; Kamano, 1965; Kono, 1968; Sato, 1974; Morton, 1981; Kato and Sakakura, 1994; Yoshio and Ishii, 1996; Mogami, 1998; Ellis and Bowers, 1998; Shimizu, 1999). All the artificial diets for butterflies developed so far contain more or less dried leaf powder of the host plant: however, it is possible to prepare the diet by mixing only chemicals and plant oils in the silkworm, *Bombyx mori* (e. g. Yushima *et al.*, 1991) and the European corn borer, *Ostrinia nubilalis* (e. g. Beck *et al.*, 1968).

Recently, ready-made diet materials for lepidopterans appeared on the market, and it is convenient to prepare the diets by mixing them with dried leaf powder of the host plant. For example, Kato and Sakakura (1994) reared larvae of the pierid, *Eurema blanda*, successfully with an artificial diet consisting of the ready-made material and dried leaf powder of some species of host plants, such as *Albizia julibrissin*. Shimizu (1999) reported that it is possible to breed and maintain the danaid, *Euploea mulciber*, in an insectarium by using an artificial diet made from dried leaf powder of the Indian oleander, *Nerium indicum*, and the ready-made material.

The chestnut tiger, *Parantica sita*, is known to migrate sometimes over 1,000 km in Japan (Fukuda, 1991), and the larvae utilize about a dozen species belonging to Asclepiadaceae as their host plants (e. g. Fukuda, 1991; Fukuda *et al.*, 1982). We are doing research to elucidate the ecological significance of the long distance migration of *P. sita*. However, it is difficult to keep providing the larvae with fresh leaves of the host plants, because most of these plants are rare in the field and/or prone to wither after collection. Furthermore, the larvae

are often parasitized by the tachinid fly, *Sturmia bella*, even in the laboratory, since it is difficult to avoid carrying the minute eggs of this tachinid with field-collected leaves (Hirai and Ishii, 1995). Thus, out of necessity, we need to develop an artificial diet for *P. sita*. In this study, we examined a mixture of a ready-made artificial diet material, and dried leaf powder of 3 plant species known as the host plants of this butterfly.

### Materials and methods

Fresh leaves of 3 species of Asclepiadaceae, *Cynanchum caudatum*, *Metaplexis japonica* and *Marsdenia tomentosa*, were collected at Nagano and Wakayama Prefectures in 1998. They were dried at 60°C immediately after collection, and ground into powder by an electric mill. We stored the powders at 5°C until the experiments took place.

We mixed the dried leaf powder with the powder of a ready-made diet material, *Insecta F-II* (Nihon-Nosan-Kogyo Co.), at the rate of 30% in dry weight for *C. caudatum* (Group CC<sub>30</sub>), *M. japonica* (MJ<sub>30</sub>) and *Ma. tomentosa* (MT<sub>30</sub>), and 10% for *C. caudatum* (CC<sub>10</sub>) and *M. japonica* (MJ<sub>10</sub>) (Table 1). As for the other preparation procedures for these artificial diets, we followed the directions for *Insecta F-II*.

Table 1. Composition of 5 artificial diets tested.

Group	Leaf powder	<i>Insecta F-II</i> powder	
CC <sub>30</sub>	<i>C. caudatum</i>	30%	70%
CC <sub>10</sub>		10	90
MJ <sub>30</sub>	<i>M. japonica</i>	30	70
MJ <sub>10</sub>		10	90
MT <sub>30</sub>	<i>Ma. tomentosa</i>	30	70
Control	Fresh leaf ( <i>Ma. tomentosa</i> )		

Eggs of *P. sita* were obtained from 8 females collected in Wakayama Prefecture on October 23–24, 1998. The newly hatched larvae were divided into 6 groups, and reared on the artificial diets or fresh leaves of *Ma. tomentosa* (the control group) under a photoperiod of 16L-8D at 19°C. Larvae were reared individually in transparent plastic petri dishes (5 cm in diameter and 1 cm in depth) and 200 ml transparent plastic cups during the 1st to 3rd instars and at the 4th and 5th instars, respectively. Pupae were kept in coupled 200 ml cups for the adult eclosion. Numbers of larvae and pupae were recorded every day, and the forewing length of the adults was measured with a plastic ruler.

### Results and discussion

Although almost all 1st instar larvae settled on the diet and adult emergence occurred in the 5 experimental groups and the control group, the mortality during the larval period varied among the groups (Table 2). No individuals died during the pupal stage in any of the groups. Rates of adult emergence in Groups MJ<sub>30</sub> and MT<sub>30</sub> were high (100 and 97%) and not significantly different from that of the control, while the larval mortality was significantly higher in Groups CC<sub>30</sub>, CC<sub>10</sub> and MJ<sub>10</sub> than that in the control (Table 2). Most of the adults observed in 4 groups, CC<sub>10</sub>, MJ<sub>30</sub>, MJ<sub>10</sub> and MT<sub>30</sub>, were healthy-looking like the control, while 25% of the adults in CC<sub>30</sub> were abnormal (mainly with crippled wings) (Table

Table 2. Numbers of individuals which eclosed into an adult and died during larval stages in the 6 groups reared on 5 artificial diets and fresh leaves (control).

Group	N	No. adult emerged (%)	No. individuals died (%)	Fischer's exact probability test <sup>a</sup>
CC <sub>30</sub>	42	36 ( 85.7)	6 (14.3)	*
CC <sub>10</sub>	30	11 ( 36.7)	19 (63.3)	***
MJ <sub>30</sub>	39	39 (100.0)	0 ( 0.0)	N. S.
MJ <sub>10</sub>	29	21 ( 72.4)	8 (27.6)	***
MT <sub>30</sub>	37	36 ( 97.3)	1 ( 2.7)	N. S.
Control	37	37 (100.0)	0 ( 0.0)	—

<sup>a</sup> Between ratio of individuals which made adult eclosion to those which died during larval stage in each experimental group and that in control: \* $p < 0.05$ , \*\*\* $p < 0.001$ , N. S.: not significant.

Table 3. Numbers of normal and abnormal adults obtained in the 6 groups reared on 5 artificial diets and fresh leaves (control).

Group	N	No. individuals (%)		Fischer's exact probability test <sup>a</sup>
		Normal	Abnormal	
CC <sub>30</sub>	36	27 (75.0)	9 (25.0)	**
CC <sub>10</sub>	11	10 (90.9)	1 ( 9.1)	N. S.
MJ <sub>30</sub>	39	38 (97.4)	1 ( 2.6)	N. S.
MJ <sub>10</sub>	21	17 (81.0)	4 (19.0)	N. S.
MT <sub>30</sub>	36	35 (97.2)	1 ( 2.8)	N. S.
Control	37	36 (97.3)	1 ( 2.7)	—

<sup>a</sup> Between ratio of individuals which made adult eclosion to those which died during larval stage in each experimental group and that in control: \* $p < 0.05$ , \*\*\* $p < 0.001$ , N. S.: not significant.

3). The survival of Group CC<sub>10</sub> was 37% and it was by a considerable margin the lowest of the 5 groups. The results suggest that the diets CC<sub>30</sub>, CC<sub>10</sub> and MJ<sub>10</sub> were not suitable for larval development in *P. sita*.

Mean larval stages of CC<sub>30</sub>, MJ<sub>30</sub> and MT<sub>30</sub> were about 26 days and were not significantly different from the control in either males or females, although CC<sub>10</sub> and MJ<sub>10</sub> were about 35 days and were significantly longer than the control (Table 3). Mean pupal stages were about 18–19 days in all the 6 groups including the control, while they were significantly longer in the males of CC<sub>30</sub>, and in the male and female of MJ<sub>30</sub> and MJ<sub>10</sub> than in the control (Table 4). Mean forewing lengths were about 55 and 57 mm for females and males, respectively, and not significantly different among all the 6 groups including the control, although females were larger than males in each group (Table 4).

From the results of this experiment, MJ<sub>30</sub> and MT<sub>30</sub> were proved to be the most suitable diets for *P. sita* among the 5 diets examined. According to Mogami (1998), pupae of the papilionid, *Atrophaneura alcinous*, reared on an artificial diet made from a mixture of dried leaf powder of *Aristolochia* spp. and *Insecta F-II* at the same composition of this study were smaller than those reared on fresh leaves of *Aristolochia* spp. by weight. By contrast, both MJ<sub>30</sub> and MT<sub>30</sub> cause neither a decrease in forewing nor a delay in larval development in *P.*

Table 4. Mean larval and pupal periods and forewing length of *P. sita* reared on 5 artificial diets and fresh leaves of *Ma. tomentosa* (control).

Group		N	Mean period $\pm$ SD (days)		Mean forewing length $\pm$ SD (mm)
			Larva	Pupa	
CC <sub>30</sub>	♂	15	25.9 $\pm$ 1.6 b	19.3 $\pm$ 1.2 a	55.4 $\pm$ 1.6 ab
	♀	12	27.2 $\pm$ 3.8 b	18.3 $\pm$ 1.1 a	57.1 $\pm$ 1.8 cde
CC <sub>10</sub>	♂	7	35.9 $\pm$ 8.4 a	18.9 $\pm$ 0.7 ae	52.6 $\pm$ 3.6 ad
	♀	3	34.3 $\pm$ 3.5 a	18.3 $\pm$ 0.6 a	55.8 $\pm$ 1.3 de
MJ <sub>30</sub>	♂	21	26.5 $\pm$ 1.8 b	19.1 $\pm$ 0.8 ac	54.6 $\pm$ 1.3 ad
	♀	17	25.8 $\pm$ 1.8 b	19.1 $\pm$ 1.1 ad	56.9 $\pm$ 1.0 cde
MJ <sub>10</sub>	♂	6	36.0 $\pm$ 6.5 a	19.2 $\pm$ 0.4 ab	52.7 $\pm$ 2.7 ae
	♀	11	35.5 $\pm$ 5.0 a	18.1 $\pm$ 0.8 bcde	55.4 $\pm$ 2.1 de
MT <sub>30</sub>	♂	25	25.9 $\pm$ 1.8 b	18.2 $\pm$ 0.8 bde	54.7 $\pm$ 1.3 a
	♀	10	25.1 $\pm$ 1.1 b	17.9 $\pm$ 0.7 bde	57.8 $\pm$ 1.2 cde
Control	♂	15	24.4 $\pm$ 1.1 b	17.7 $\pm$ 0.6 e	54.9 $\pm$ 1.8 ac
	♀	21	24.9 $\pm$ 1.2 b	17.8 $\pm$ 1.0 e	56.2 $\pm$ 1.5 bcde

Values with the same letter in the same column are not significantly different by Tukey-Kramer's test ( $p > 0.05$ ).

#### *sita*.

*Ma. tomentosa* is known as one of the most common host plants of *P. sita* in Japan (e. g. Kanazawa *et al.*, 1993; Hirai and Ishii, 1997), although the distribution is relatively restricted with each colony being quite small. By contrast, *M. japonica* is common in grasslands and on the roadside, with each colony usually being large from summer to autumn. Endo and Nihira (1990) considered that *M. japonica* is available for a larval diet of *P. sita* only in the laboratory. On the other hand, Ohara (1999) reported that *P. sita* larvae were found feeding on this plant in Tokushima Prefecture, and indeed we also found eggs and a few larvae, including a full-grown larva on this plant in Nara and Nagano Prefectures, respectively. Thus, an artificial diet made from *M. japonica* seems to be the most convenient for mass rearing of *P. sita*.

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#### References

- David, W. A. L. and B. O. C. Gardiner, 1965. Rearing *Pieris brassicae* L. on a semi-synthetic diet. *Nature* (207): 882-883.
- Ellis, A. and M. D. Bowers, 1998. Effects of hostplant species and artificial diet on growth of buckeye (*Junonia coenia*) and painted lady (*Vanessa cardui*) caterpillars (Nymphalidae). *J. Lepid. Soc.* **52**: 73-83.
- Endo, S. and I. Nihirai, 1990. *Larval Food of Japanese Butterflies*. 74 pp. Ginzainsatu, Tokyo. (In

- Japanese).
- Fukuda, H., 1991. Seasonal migration of the chestnut tiger, *Parantica sita* in Japan. *Insectarium* **28**: 384-393 (in Japanese).
- Fukuda, H., Hama, E., Kuzuya, K., Takahashi, A., Takahashi, M., Tanaka, B., Tanaka, H., Wakabayashi, M. and Y. Watanabe, 1982. Papilionidae, Pieridae, Danaidae. *The Life Histories of Butterflies in Japan* **1**. xxii, 65-277 pp., 64 pls. Hoikusha, Osaka. (In Japanese).
- Hirai, N. and M. Ishii, 1995. Host relationships of the tachinid fly, *Sturmia bella*, and the trigonalid wasp, *Poecilognathos fasciata*, parasitizing the chestnut tiger butterfly, *Parantica sita*. *Appl. Ent. Zool.* **30**: 241-244.
- , 1997. Seasonal occurrence of the chestnut tiger butterfly, *Parantica sita* (Lepidoptera: Danaidae), at 3 habitats in the Kii Peninsula, central Japan. *Trans. lepid. Soc. Japan* **48**: 223-233.
- Kato, Y. and S. Sakakura, 1994. Artificial diet rearing of *Eurema blanda* and some notes on its host-plant. *Tyô Ga* **45**: 21-26 (in Japanese with English summary).
- Kamano, S., 1965. Rearing of *Papilio xuthus* larvae on artificial diets. *Jap. J. appl. Ent. Zool.* **9**: 133-135 (in Japanese).
- Kanazawa, I., Okuno, S., Takemoto, T., Miyatake, Y., Hosoi, T. and M. Ohtsuki, 1993. Bionomics of the chestnut tiger, *Parantica sita* (Lepidoptera, Danaidae) in the western part of Japan—Results of researches from 1983 to 1989 by The Chestnut Tiger Research Group. *Bull. Osaka Mus. nat. Hist.* **47**: 47-59.
- Kono, Y., 1968. Rearing *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae) on artificial diets. *Appl. Ent. Zool.* **3**: 96-98 (in Japanese).
- Mogami, E., 1998. Rearing lepidopterous larvae on artificial food at Tama Zoo. *Insectarium* **35**: 188-193 (in Japanese).
- Morton, A. C., 1981. Rearing butterflies on artificial diets. *J. Res. Lepid.* **18**: 221-227.
- Ohara, K., 1999. Note on the natural foodplant of *Parantica sita* (Lepidoptera: Danaidae). *Bull. Tokushima Pref. Mus.* **9**: 57-60 (in Japanese).
- Shimizu, S., 1999. Breeding of the cramer striped blue, *Euploea mulciber*, at the Osaka Prefectural Minoo Park Insectarium. *Insectarium* **36**: 364-365 (in Japanese).
- Sato, Y., 1974. Rearing *Pieris rapae crucivora* larvae on artificial diets. *Kontyû* **42**: 467-472 (in Japanese with English summary).
- Yoshio, M. and M. Ishii, 1996. Rearing larvae of the Mormon butterfly, *Papilio memnon* L. (Lepidoptera: Papilionidae), on artificial diet. *Jap. J. Ent.* **64**: 30-34.
- Yushima, T., Kamano, S. and Y. Tamaki (Eds), 1991. *Rearing Methods of Insects*. 392 pp. Japanese Society of Plant Protection, Tokyo. (In Japanese).

## 摘 要

アサギマダラ幼虫の人工飼料による飼育 (平井規央・石井 実)

アサギマダラ *Parantica sita* を市販の原体 (日本農産工業社 (株) 製インセクタ F-II) にイケマ *Cynanchum caudatum*, ガガイモ *Metaplexis japonica*, キジョラン *Marsdenia tomentosa* (ガガイモ科) の乾燥葉粉末を 30% または 10% 加えた 5 種類の人工飼料で飼育した。すべての人工飼料で成虫が羽化したが、イケマ 10% 区およびガガイモ 10% 区では、キジョラン生葉 (対照区) と比較して幼虫期間が長かった。また、イケマ 30% 区、イケマ 10% 区およびガガイモ 10% 区の人工飼料では対照区と比較して幼虫期の死亡率が高く、さらに、イケマ 30% 区では翅が正常に伸びない個体の割合が対照区よりも高かった。ガガイモ 30% 区およびキジョラン 30% 区では、幼虫期間、死亡率、成虫のサイズは対照区と有意差はなく、入手の容易さを考慮すると、ガガイモを利用した人工飼料が本種の大量飼育にもっとも適していると考えられる。

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